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DITERPENE CONSTITUENTS OF *JUNIPERUS POLYCARPOS* AND THEIR ANTIMICROBIAL AND ANTI-INFLAMMATORY ACTIVITIES

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ABSTRACT

Activity-directed study of *Juniperus polycarpos* led to the isolation of active antimicrobial viz., hinokiol, sandaracopimaric acid, 4-epiabietic acid, and other minor terpenes in addition to sabinic acid. The antiinflammatory activity of hinokiol was also demonstrated using carrageenan-induced inflammation in rats. The structures of the isolated terpenes were determined by ^{1}HNMR, ^{13}CNMR and 2DNMR, APT, DEPT, PND spectra.

INTRODUCTION

Members of the genus *Juniperus* (Family Cupressaceae) are ever green shrubs or trees. This genus comprises about 60 species distributed in the northern hemisphere[1]. Some species of *Juniperus* are economically important for wood production e.g *J.virginiana* (for making pencils, red cedar wood) others e.g. *J. polycarpos*, *J. procera* (as fuel and for building constructions). Some *Juniperus* volatile oils are known medicinal e.g oil of savin (counter irritant), oil of cade (for skin diseases), oil of Juniper (diuretic, antiseptic) and also in making certain varieties of gin [1]. Many world wide *Juniperus* species are recently reported for antimicrobial activities of their essential oils and terpenoid constituents[2-5]. Other *Juniperus* are reported for antifertility[6], antiherpatic (for HSV-1)[7,8], antitumor (P- 388) [9] and for treatment of facial lesions [10].

Juniperus polycarpos is an ornamental tree commonly cultivated for shade or wind break and its leaves and barks are traditionally recommended for wounds as dressing with other plant recipes. However, the constituent(s) responsible for such folkloric use still undiscovered. The essential oil of the leaves of *J. polycarpos* has been analysed and found rich with terpene hydrocarbons, alcohols and ketones[11].

EXPERIMENTAL

Plant material:

Leaves and barks of *Juniperus polycarpos* Auct. E. Koch. (Family Cupressaceae) were collected from Addis Ababa University Campus and Kindly identified by Dr. Getachew Worku, Herbarium Department, College of Science, Addis Ababa University, Ethiopia.

Methods:

Melting points were uncorrected. ^{1}H and ^{13}CNMR 200 and 300 and 75 MHz respectively were run in $CDCl_3$, or DMSO-d_6,(using TMS as internal standard) using Varian XL200 or VXR instruments. COSY, HETCOR, DEPT, and APT spectra were recorded. EIMS: 70 eV. Column chromatography, TLC, Silica gel, solvent (Hexane: ether) mixture and visualization by 1% vanillin: H_2SO_4 spray reagent.

Antimcrobial and anti-inflammatory activities:

The antimicrobial activity of the different extracts and pure compounds were performed on standard testing organisms (*B. subtilis* NCTC # 10400, *Staph. aureus* NCTC # 6571, *Strept. durans* NCTC # 8307) using the disc standard procedure[12] (Table 1). Anti-inflammatory activity was performed using carrageenan-induced inflammation in rats previously described[13].

The volatile oil of leaves of *J. polycarpos* was prepared by hydrodistillation of fresh leaves (0.1 %), dried over anhydrous sodium sulfate and then used for testing against microbes (Table 1).

Extraction and Isolation of Constituents:

Juniperus Polycarpos air-dried powdered bark (3 kg) were exhaustively extracted with chloroform using a continuous extraction apparatus. Evaporation of the solvent (*in vacuu*) yielded ~315 g dry residue. A portion of the dry extract (~ 186 g) was partitioned between acetonitrile and hexane led to the separation of amorphous materials (~ 19 g) at the interphase of crude sabinic acid (**1**).

The acetonitrile fraction yielded on concentration (*in vacuu*) and crystallization from the same solvent an amorphous material (1.4g). This material was column chromatographed on silica gel (50g) using hexane as developer and the polarity was then increased by gradual addition of ether up to 50 %. The eluates afforded ~300 mg of pure hinokiol (**2**) after crystallization from acetonitrile. Further increase of the polarity of solvent by addition of ether to hexane (ether: hexane, 3:1) led to isolation of compound (**3**) [R_f 0.54, TLC using ether: hexane, 1:1].

Compounds contained in the hexane partition fraction (8 g) were separated on column chromatography (200 g) using silica gel (E. Merck) and eluted with hexane then hexane: ether mixtures. Fatty

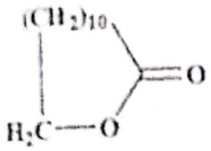
materials were first eluted with hexane and the more polar compounds (only fractions) were eluted out with hexane : ether (1:1) mixture. Repeated chromatography of the only fractions using the same solvents led to the isolation of 4-epiabietic acid (**4**, 90 mg) as the major diterpene constituent and sandaracopimaric acid (**5**, 30 mg) m.p 160°C. Another minor component, 4-epi-7(8)-dehydroabietic acid (**6**) was isolated when compound (**4**) enriched fractions were rechromatographed on silica gel column (20 g) impregnated with 10% $AgNO_3$. Elution of this minor constituent was affected by hexane:tetrahydrofuran (9.5 : 0.5) mixture after separation of (**4**) and other impurities.

12-hydroxylauric acid (sabinic acid) (**1**):

This compound was crystalized from CH_3CN to give lactone ($C_{12}H_{22}O_2$) with m.p 78°C, $[\alpha]^{25}D$+98 ($CHCl_3$, c= 0.1). IR $\sqrt{}_{max}$ (KBr) 2840, 1730, (ester C=O), 1460, 1370 cm^{-1}. ^1HNMR δ_{ppm} ($CDCl_3$) 4.076 (1H, CH-O, t, J=7 Hz), 2.31 (1H, CH-O, t,7.2Hz), 1.6 (m,CH_2); 1.28 (br. s,CH_2). $^{13}CNMR$ δ_{ppm} ($CDCl_3$) [25.03, 25.95,28.67,29.19, 29.27, 29.50, 29.54, 29.61, 29.66-CH_2] 34.4 (CH_2-CO),64.41 (CH_2-O), 174.02 (lactone C=O) indicated by PND, DEPT, APT spectra.

Hinokiol (2):

This compound showed $[\alpha]^{25}D$+107.9 ($CHCl_3$: c= 0.1), m.p 241°C; IR $\sqrt{}_{max}$ (KBr): 3254 (OH), 3300, 1620, 1520, 1475, 1425 cm^{-1}. EIMS m/z: 302 (M^+ = $C_{20}H_{30}O_2$), 287 (14%, M^+-CH_3), 270 (20%), 243, (19%, M^+-CH_3-CO_2), 257 (2.4%, M^+-isopropyl), 227(48%), 216 (10%), 215 (55%), 213(21%), 203(13%), 202(37%), 201 (48%), 200 (18%), 199 (85%); $^1HNMR\delta_{ppm}$ ($CHCl_3$):3.3 (H-3, dd, J=4.7, 5.4 Hz), 4.5 (br. s, OH), 1.73 (H-5, d, J=12 Hz), 1.77-1.80 (H-6, m), 2.76-2.82 (H-7, m), 6.83 (H-11, s), 6.612 (H-14, s), 2.84 (H-15, m),1.20 (H-16, d,J=6.9 Hz) 1.22 (H-17, d, J=6.9 Hz), 0.88,(H-18, s),1.06 (H-19, s),1.16 (H-20, s). $^{13}CNMR$: Table (2) indicated by PND, APT, DEPT spectra and literature[4].



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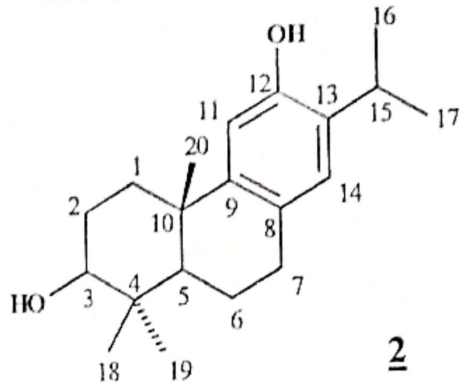

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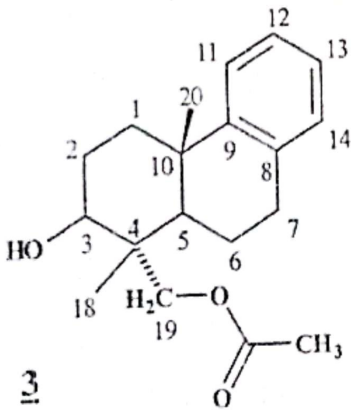

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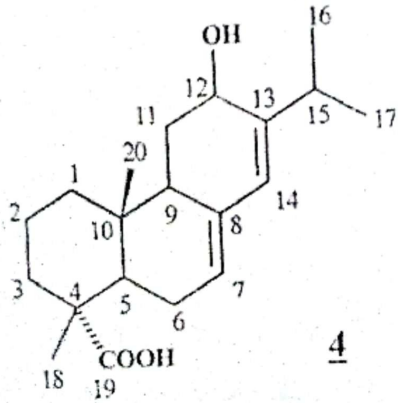

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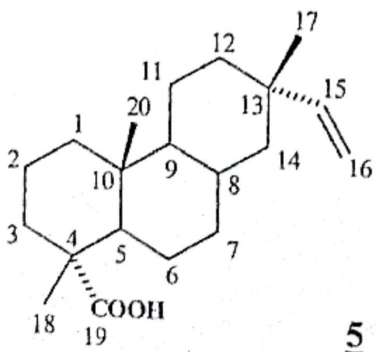

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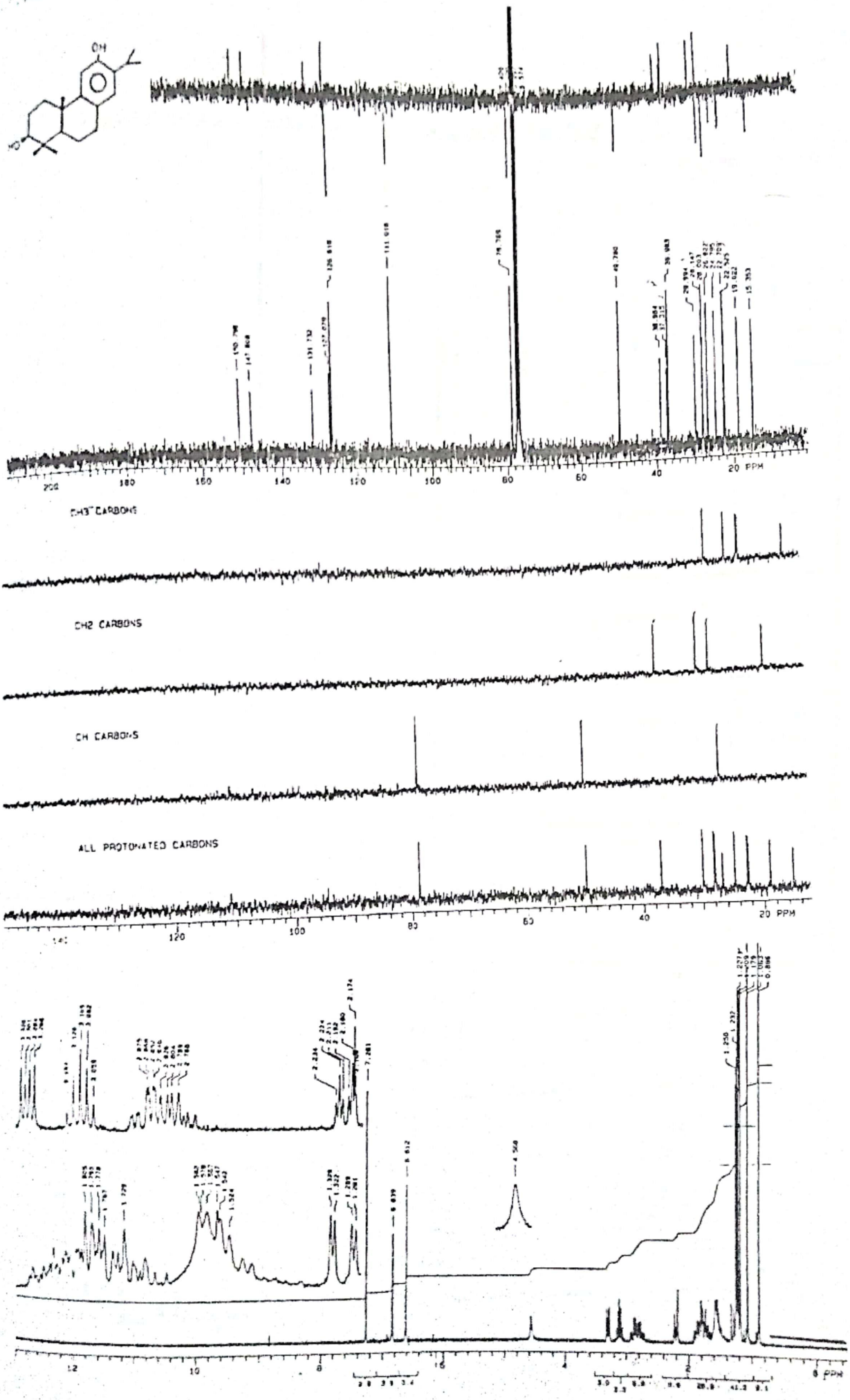
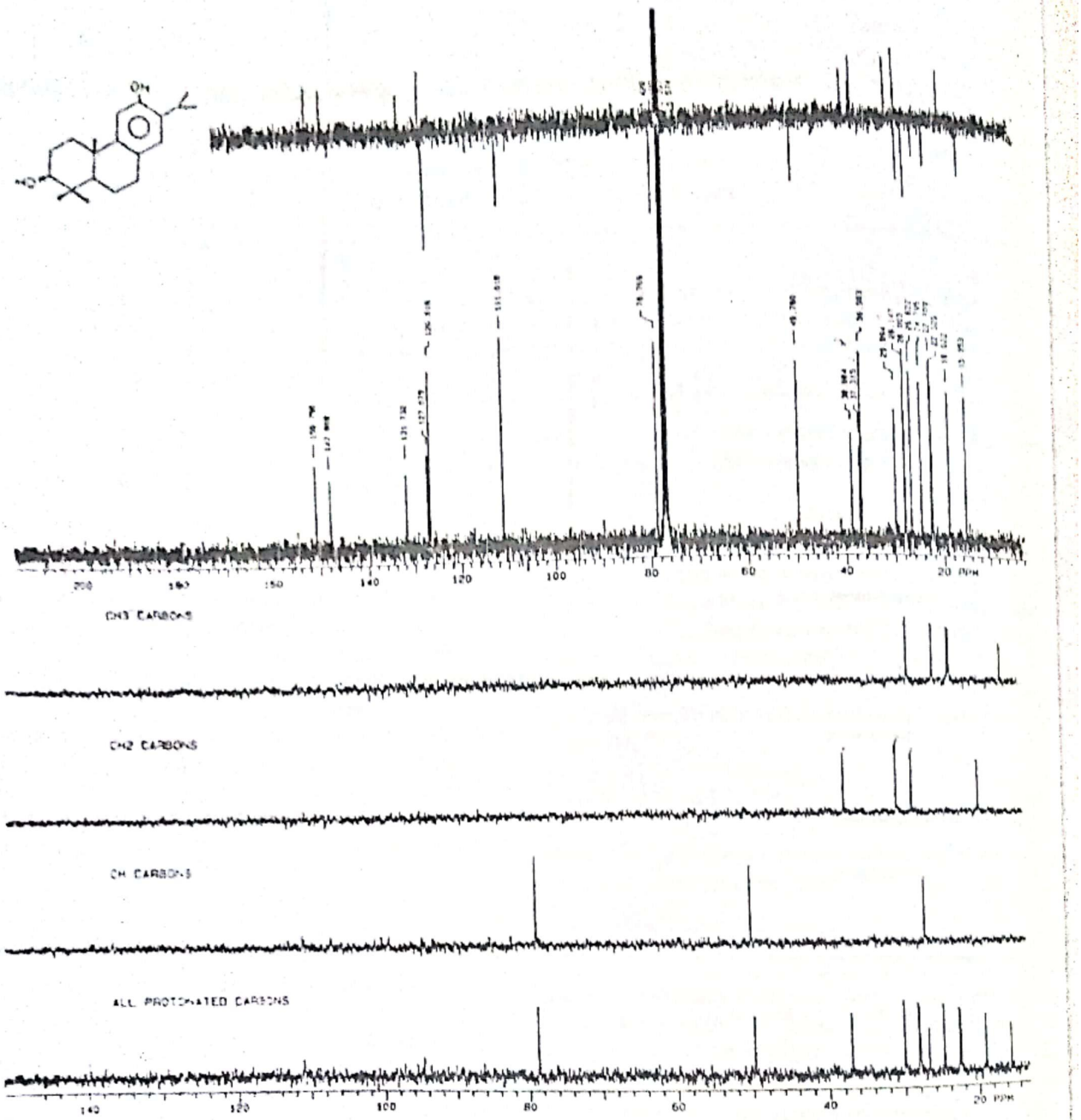

OH
HO
CH3 CARBONS
CH2 CARBONS
CH CARBONS
ALL PROTONATED CARBONS
20 PPM
PPM


CH3 CARBONS
CH2 CARBONS
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ALL PROTONATED CARBONS
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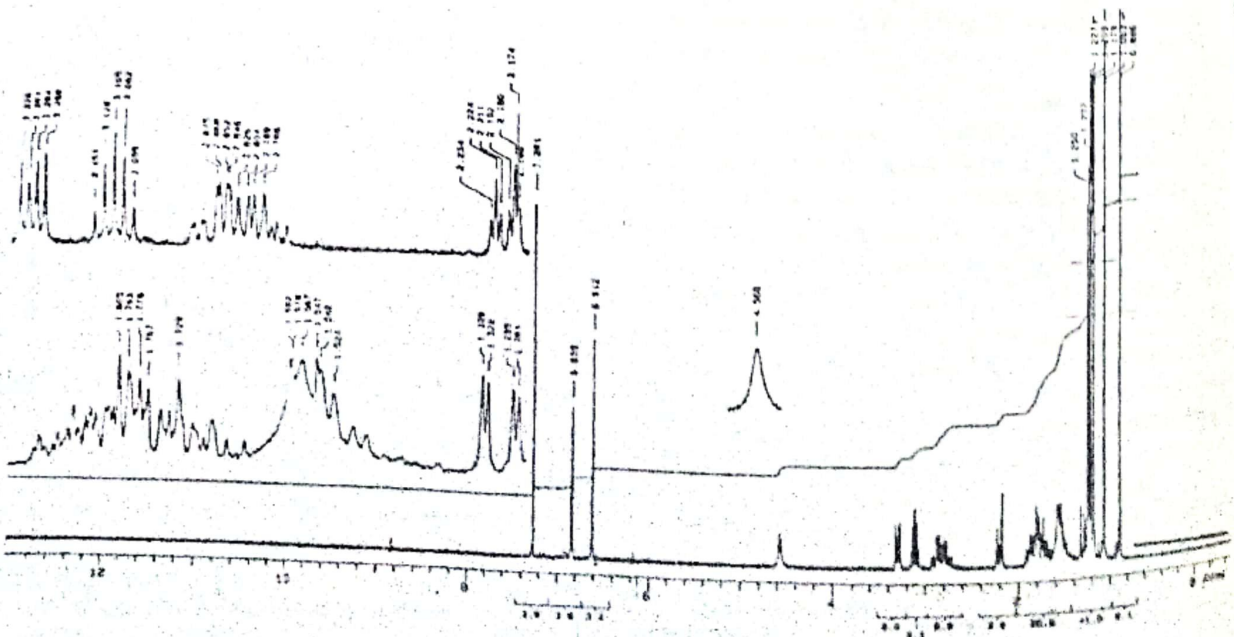

Compound (3):

Degraded abietane, showed ^{1}HNMR δ_{ppm} (DMSO-d_6): 0.93 (CH_3, s), 1.08 (CH_3, s) 2.55 (CH_3, s), 2.67 (CH_2), 4.5 (CH-O, t), ~7.38 (4 Ar-H, m), 8.2 (br. s, OH). ^{13}CNMR δ_{ppm} (DMSO-d_6): 38.7 (C_1), 19.5 (C_2), 69.5 (C_3-OH), 43.32 (C_4), 56.49 (C_5), 34.43 (C-), 28.4 (C_8), 141.5 (C_9), 43.32 (C_{10}), 126.1 (C_{11}), 128.4 (C_{12}), 126.5 (C_{13}), 127.4 (C_{14}), 169.83 (acetyl C=O), 69.77 (C_{15}-CH_2O), 30.81 (C_{16}-CH_3), 9.4 (C_{17}-CH_3), indicated by PND spectrum.

8-epiabietic acid (4):

This compound showed $[\alpha]^{25}$D-1.25 (EtOH: c= 1.5) IR ν_{max} (KBr): 3450(OH), 1695, 1460, 1450, 1385 cm^{-1}. ^{1}HNMR δ_{ppm} ($CDCl_3$): 0.71 (H-20, CH_3, s), 1.24 (H-18, CH_3, s), 1.02 (H-16, H-17, $2CH_3$, d, J= 6.9Hz), 4.78 (H-14 s), 5.4 (H-7, m), 11 (br. s, OH). ^{13}CNMR ($CDCl_3$) Table (2) indicated by PND, APT, DEPT spectra and literature[15].

Sandaracopimaric acid (5):

This compound showed m.p. 161 °C. IR ν_{max} (KBr): 1700 (C=O), 1470, 1420, 1285 cm^{-1}. ^{1}HNMR δ_{ppm} (DMSO-d_6): 1.21 (H-18, CH_3, s) 1.04 (H-20, CH_3, s) 0.84 (H-17,CH_3, s), 3.22 (H-14, s), ~4.9 (H-16$_a$ d, J=15 Hz), ~5.8 (H-16$_b$, d, J= 10 Hz) 4.8 (H-15, dd, J=10.5, 15Hz). ^{13}CNMR (Table 2) supported by HETCOR, COSY, APT, DEPT and PND spectra and literature[16].

Compound (6):

This compounded showed ^{1}HNMR δ_{ppm} ($CDCl_3$): 0.65 (H-20, CH_3, s), 1.25 (H-18- CH_3, s), 1.75 (H-17, CH_3, s), 6.3 (H-7, dd, J=10.8 Hz), 5.07 H-16 CH_2, m). ^{13}CNMRδ_{ppm} ($CDCl_3$): 38.49 (C_1), 19.93 (C_2), 40.37 (C_3), 44.23 (C_4), 56.3 (C_5), 23.31 (C_6), 141.59 (C_7), 133.88 (C_8), 56.42 (C_9), 39.9 (C_{10}), 25.82 (C_{12}), 147.89 (C_{13}), 133.88 (C_{14}), 133.43 (C_{15}), 11.84 (C_{16}), 109.89 (C_{17}), 29.4 (C_{18}), 184.44 (C_{19}), 12.83 (C_{20}), supported by PND, APT.DEPT, spectra.

RESULTS AND DISCUSSIONS

Many diterpenoids with various skeletal structures have been reported from non-volatile constituents of *Juniperus* plants[14-17]. However, the isolation of 12-hydroxylauric acid in a considerable amount (0.63%) among the non-terpene resin components may contribute with the volatile oil for the antibacterial activity of the chloroform extract. Hinokiol **2**, the major abietane constituent of this plant has been reported previously in species of *Chamaecyparis*, *Cupressus* and *Juniperus*[18- 20]. The demonstrated antibacterial activity (Table 1) and reduction of inflammation (Table 3) induced by carrageenan in rats (8.4%) may explain the traditional use of this plant for infected wounds.

The ^{1}HNMR and ^{13}CNMR spectra of this compound (Fig. 1) were typical of abietane skeleton, and comparable with the literature data of hinokiol[21]. The oxymethine proton at 3.3 ppm (dd, J= 4.7, 5.4 Hz) suggests an axially disposed C_3-OH group.

Table (1): ^{13}CNMR Chemical Shift Values of Isolated Diterpenes.

Carbon	2	4	5
1	28.1	39.22	38.4
2	36.9	19.97	18.5
3	78.7	35.40	37.03
4	38.9	43.87	47.2
5	49.78	51.51	48.7
6	19.2	23.05	24.9
7	29.9	121.22	35.46
8	127.1	134.43	136.6
9	147.8	49.99	50.57
10	37.3	38.15	37.73
11	111.0	24.64	18.57
12	150.1	27.50	34.44
13	131.7	144.9	37.40
14	126.0	122.39	129.13
15	26.8	34.83	148.89
16	22.7	20.87	110.14
17	22.52	21.43	26.03
18	15.35	28.87	16.78
19	28.0	184.9	184.71
20	24.79	12.73	15.21

Table (2): Antimicrobial Activity of *Juniperus Polycarpos*.

Material	Conc. µg/ml	*S. aureus*	*B. subtilis*
Essential oil	125	+	+
Chloroform extract	125	+	+
Hexane extract	62	+	+
Acetonitrile extract	62	+	+
Abietic acid	120	-	+
Sabinic acid lactone	125	-	+
Sandaracopimaric acid	180	-	+
Hinokiol	12	+	+

Table (3): Antiinflammatory Activity of Hinokiol on Carrageenan- Induced Inflammation in Rats.

GROUP	Dose mg/ kg body weight orally	Volume of Paw (ml) after Carrageenan administration (Mean ± S.E.)			Total increase in paw volume(ml) after three hours	percent inhibition
		0 hour	1 hours	2 hours	3 hours	
CONTROL (6)*	-	1.03 ± 0.015	1.576 ± 0.015	1.76 ± 0.012	1.81 ± 0.015	0.71 ± 0.04	-
Test (6)*	100	1.46 ± 0.013	1.60 ± 0.015	1.81 ± 0.02	1.87 ± 0.015	0.65 ± 0.06	8.4

* Figures in parentheses denote the number of animals.

The minor component 3 separated from hinokiol containing fractions showed 17 skeletal carbons (six aromatics, two methyls and two oxycarbons and one acetate residue 169.8 C=O). The chemical shift values of its carbons were in agreement with a degraded abietane. The HNMR spectrum also showed methyl singlets at 0.93, 1.08 ppm, four aromatic protons ~ 7.38 ppm, a hydroxyl at ~ 8.2 ppm and oxymethylene ~ 4.5 ppm. These data suggested the tentative structure 3 to this isolate.

The carbon skeleton of the pimarane diterpene 5 was prepared on the basis of ^{13}CNMR data (Fig. 2). The presence of three methyls, four olefenic carbons (110.1, 129.1, 136.6, 148.8 ppm and acid carbonyl ~ 184.7 ppm are in accord with this terpene acid functionalities. ^{1}HNMR spectrum (Fig. 2) also confirmed this assignment and showed methyl singlets at 0.84, 1.04, and 1.21 ppm. Methine proton at 5.8, methylene protons at ~ 4.8 ppm indicative of a group (-HC=CH_2) and in addition to olefinic singlet at 5.2 ppm all are comparable to reported data of sandaracopimaric acid previously reported in *J. communis*[22].

The recent discovery of its inhibitory activity on the enzyme 15-lipoxygenase and subsequent leukotriene synthesis[23] may prove that it contributes to the anti-inflammatory activity of this crude drug. Sandaracopimaric acid obtained from *J. sabina* also evidenced tumor inhibitory activity on animal models[9].

The terpene acid 4 showed spectral data specifically correlated with abietic acid[15]. The stereochemistry of C-18 methyl of 4 and 6 were assigned as the 4-epi-isomer by the shift value correlation ~ 1.24, 1.25 ppm with previously reported data[24]. Although the available data on 6 may tentatively suggest a structure similar to 15, 17-dihydrogabietic acid; reisolation of extra amount of this compound may be necessary to confirm these results.

REFERENCES

1. Trease and W.C. Evan's ; Pharmacognosy; 14th ed. WB Saunders company LTD, 1996.
2. Bogei, E., Digrak, M., **Turk J. Biol.**, 20, 191-198, (1996).
3. Stossi, V.; Veny Kokidou, E.; Lowise, A. ; **Flavour Fragrance J.,** 11, 71-4, (1996).
4. Muhammad, I.; Mossa, J.S.; EL-Feraly, F.S; **Phytother. Res.** 6 (5), 261-4, (1992).
5. Muhammad, I.; Mossa, J.S.; Al-Yahya; M.A; Ramadan, A.F; EL-Feraly, F.S.: **Phytother. Res.,** 9(8), 584, (1995).
6. Pana Kash, A., O. : **Int. J. Crude Drug Res.,** 21(1), 19-24, (1986).
7. Markkanen T. : **Drugs Exp. Clin. Res.,** 7(1), 53-6, (1981).
8. Markkanen, T.; Makinen, M.L.; Nikoskelainen, J; Ruohonen, J.; Meninen, K.; To- Kinen, P.; Kenwnio, P.; Hirvonen, T.: **Drugs Exp. Clin. Res.** 7(1), 691-7, (1981).
9. Feng, S., G., Yunlong; yu, H; Mu sadillon, S., **Zhiwu xuebao,** 31(5), 382-8 (1989). Via **Chem. Abst** 112, (1990).
10. Walsh, W., E., PCT Int. Appl. Wo 88091794, 1988.
11. Chavchanidze, V.Y., Kharabava, L.G.: Subtrop. Kul't., (4), 131-43 (Russ.) 1989. Via **Chem. Abst.** Vol. 112, (1990), 213884 J.
12. Barry, A.L. : The Antimicrobial Suscept Test Principles and Practice. Lea and Feblgir, Philadelphia, (1976).
13. Winter, C.A. Risley, E.A. , and Nuss, G.W., **Proc. Soc. Exp. Biol. Med.** 111, 544, (1962).

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Vol. 7, No. 1, pp. 80-86

13. Winter, C.A. Risley, E.A. , and Nuss, G.W., **Proc. Soc. Exp. Biol. Med.** 111, 544, (1962).

14. Kuo, Yueh-Hsiung; yu, mong, T.Sang: **Phytochemistry**, 42(3) 779, (1996).

15. San feliciano, Arturo; Caballero, Esther; Del. K. Benedicts; sancho Isabel: **Phytochemistry**, 30(9), 3134; (1991).

16. Kuo, Yueh-Hsiung; Yu, Wing-Tsang: **Pharm. Bull.**, 44(6), 1242, (1996).

17. Barreto, H.S. and C. Bengell: **Acta Chem. Scand.**, 15, 1313, (1961).

18. Dawidar, A.M., Abdel-Mogib, M.; E. Zwirly, T.S.: **Pharmazie** 46 (6) 472, (1991).

19. Berkman, M.Z.; Sakar, M.K.; San Feliciano, A. **Acta Pharm. Turc.**, 36 (3), 64, (1994).

20. Yoshiki, Y. and T. Ishiguro: **Yakugaku Zasashi**, 53, 73, (1933).

21. Christiane, T. and Claude, P.: **Phytochemistry**, 10, 1639, (1971).

22. Wenkert, E.; G. Vernon Baddelay; Ivor R. Burfitt and Louis N. Morens: **Org. Mag. Res.** 11(7), (1978).

23. Depascaul Teresa, J.; San felicians, A.; Barrero, A.F.: **An. Quim.**, 69(9-10), 1065, (1973).

24. Comte, G.; Alavs, D.P.; Simon, A.; Esaady, D.; Chulia, A. J.; Delage, C.; Sauk, W.: **J. Nat. Prod.**, 58(2), 238, (1995).

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المكونات التربينية لنبات جونيبيرس بولي كاربس وتأثيرها المضاد للميكروبات والإلتهابات

على محمد السيد

قسم العقاقير – كلية الصيدلة – بقصر العينى – جامعة القاهرة – مصر

باتباع الدراسة الموجهة للفاعلية على نبات الجينوبيرس بوليكاربوس، تم فصل المواد المهبطة للميكروبات مثل الهينوكيول وحامض ساندركوبيمارك، وحامض ابيتك إلى جانب تربينات أخرى وحامض السابينك. وقد وجد للهينوكيول تأثير معالج للإلتهابات إلى جانب تأثيره على الميكروبات.

وقد تم استنباط التركيب البنائى لهذه المركبات باستعمال الطرق الطيفية المختلفة.